

What is claimed is:

1. A method for identifying at least one peptide or protein in a sample, comprising:
contacting the sample to a solid surface, wherein the solid surface comprises an affinity-binding molecule of the at least one peptide or protein;
washing the solid surface using a mobile phase to produce at least one eluent, wherein the eluent comprises the at least one peptide or protein;~~and~~
characterizing the at least one peptide or protein in the at least one eluent using a mass spectrometer under native conditions;
- ~~2. The method of claim 1, further comprising~~ generating at least one separation profile;
- ~~wherein the at least one peptide or protein is a drug, an antibody, a bispecific antibody, a monoclonal antibody, a fusion protein, an antibody-drug conjugate, an antibody fragment, or a protein pharmaceutical product; and~~
~~quantifying a drug-to-antibody ratio of the antibody-drug conjugate based on the at least one separation profile or a comparison with another separation profile.~~
- ~~23.~~ The method of claim ~~21~~, further comprising identifying or quantifying the at least one peptide or protein based on the at least one separation profile.
- ~~43.~~ The method of claim ~~21~~, further comprising identifying or quantifying a level of post-translational modification or post-translational modification variation of the at least one peptide or protein based on the at least one separation profile or a comparison with another separation profile.
- ~~54.~~ The method of claim ~~21~~, further comprising identifying or quantifying a level of glycosylation or glycosylation variation of the at least one peptide or protein based on the at least one separation profile or a comparison with another separation profile.
- ~~65.~~ The method of claim ~~54~~, wherein the glycosylation is terminal galactose, Fc glycan occupancy, core fucose, bisecting GlcNAc, or Man5.

~~76.~~ The method of claim ~~21~~, further comprising separating or identifying an impurity in the sample based on the at least one separation profile or a comparison with another separation profile.

~~8.~~ ~~The method of claim 2, wherein the at least one peptide or protein is a drug, an antibody, a bispecific antibody, a monoclonal antibody, a fusion protein, an antibody-drug conjugate, an antibody fragment, or a protein pharmaceutical product.~~

~~9.~~ ~~The method of claim 8, further comprising quantifying a drug-to-antibody ratio of the antibody-drug conjugate based on the at least one separation profile or a comparison with another separation profile.~~

~~107.~~ The method of claim 1, wherein the solid surface comprising the affinity-binding molecule of the at least one peptide or protein is included in a chromatography column.

~~118.~~ The method of claim ~~107~~, wherein the mass spectrometer is coupled online to the chromatography column.

~~129.~~ The method of claim 1, wherein the affinity-binding molecule of the at least one peptide or protein is protein A, protein G, Fcγ receptor, FcγRIIIa, anti-human Fc antibody, neonatal Fc receptor, Fc epsilon RI, anti-idiotypic antibody, or complement component C1q.

~~1310.~~ The method of claim ~~107~~, wherein a splitter is used to connect the mass spectrometer and the chromatography column.

~~1411.~~ The method of claim ~~1310~~, wherein the splitter is used to divert a low flow to the mass spectrometer and a high flow to a detector.

~~1512.~~ The method of claim 1, wherein the mobile phase is an acidic solution and the eluent is characterized using the mass spectrometer under native conditions without pretreatment.

~~1613.~~ The method of claim 1, wherein the mobile phase comprises ammonium acetate, acetic acid, or a combination thereof.

~~17~~14. The method of claim ~~10~~7, wherein the mobile phase is used to wash the chromatography column and has a flow rate of ~~about~~ 0.2-0.6 mL/min.

~~18~~15. The method of claim 1, wherein the mass spectrometer is an electrospray ionization mass spectrometer, nano-electrospray ionization mass spectrometer, a triple quadrupole mass spectrometer, a quadrupole mass spectrometer or a ultra-high mass range hybrid quadrupole mass spectrometer.

~~19~~16. The method of claim 1, wherein the mass spectrometer comprises an orbitrap mass analyzer.

~~20~~17. A system for identifying at least one peptide or protein, comprising:

a sample comprising the at least one peptide or protein;

a chromatography column comprising an affinity-binding molecule of the at least one peptide or protein, wherein the chromatography column is capable of being washed by a mobile phase to generate an eluent; ~~and~~

a mass spectrometer capable of characterizing or quantifying the at least one peptide or protein, wherein the mass spectrometer is capable of being run under native conditions, and being coupled online to the chromatography column; ~~and~~

generating at least one separation profile;

wherein the at least one peptide or protein is a drug, an antibody, a bispecific antibody, a monoclonal antibody, a fusion protein, an antibody-drug conjugate, an antibody fragment, or a protein pharmaceutical product; and

further comprising quantifying a drug-to-antibody ratio of the antibody-drug conjugate based on the at least one separation profile or a comparison with another separation profile.

~~21~~18. The system of claim ~~21~~17, wherein a splitter is used to connect the mass spectrometer and the chromatography column.

~~22~~19. The system of claim ~~21~~18, wherein the splitter is used to divert a low flow to the mass spectrometer and a high flow to a detector.

[2320](#). The system of claim [2017](#), wherein the mobile phase is an acidic solution and the eluent is characterized using the mass spectrometer under native conditions without a pretreatment.

[2421](#). The system of claim [2017](#), wherein the mobile phase comprises ammonium acetate, acetic acid, or a combination thereof.

[2522](#). The system of claim [2017](#), wherein the mobile phase has a flow rate of ~~about~~ 0.2-0.6 mL/min.

[2623](#). The system of claim [2017](#), wherein the system comprises a diode-array detector or a photodiode array detector.

[2724](#). The system of claim [2017](#), wherein the at least one peptide or protein is a drug, an antibody, a bispecific antibody, a monoclonal antibody, a fusion protein, an antibody-drug conjugate, an antibody fragment, or a protein pharmaceutical product.

[2825](#). The system of claim [2017](#), wherein the mass spectrometer is an electrospray ionization mass spectrometer, nano-electrospray ionization mass spectrometer, a triple quadrupole mass spectrometer, or a ultra-high mass range hybrid quadrupole mass spectrometer.

[2926](#). The system of claim [2017](#), wherein the mass spectrometer comprises an orbitrap mass analyzer.

[3027](#). The system of claim [2017](#), wherein the affinity-binding molecule of the at least one peptide or protein is protein A, protein G, Fcγ receptor, FcγRIIIa, anti-human Fc antibody, neonatal Fc receptor, Fc epsilon RI, anti-idiotypic antibody, or complement component C1q.